



Restoration of the viability and bioactivity of endophytic fungi using epigenetic modification

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ABSTRACT

The growing threat of antibiotic resistance has prompted investigations into alternative sources of antibacterial agents. Endophytic fungi have emerged as a promising source of a variety of potent bioactive compounds. However, their bioactivity and viability often diminish with extended storage and cultivation. In this study, two endophytic fungi were screened for their viability and antibacterial potential through successive subculture and agar diffusion techniques. Epigenetic modification and combination therapy were adopted to reverse the antibacterial potential. *Aspergillus* sp. isolate UILRZ5 became nonviable at the second generation, while *Penicillium citrinum* isolate UILRZ6 remained partially viable at the third generation. The first-generation activity ranged between 7.00 ± 0.00 mm to 13.50 ± 0.50 mm, while the minimum inhibitory concentration (MIC) ranged between 32 and 256 $\mu\text{g/mL}$. Nicotinamide was used as an epigenetic modifier to reactivate the lost antibacterial activity to a range of 3.70 ± 0.25 mm to 9.33 ± 0.21 mm and MIC between 32–256 $\mu\text{g/mL}$. The combination approach, with the inclusion of penicillin, had a zone of inhibition between 2.20 ± 0.20 mm and 19.23 ± 0.25 mm. The Gas chromatography-mass spectrometry analysis detected several chemical compounds of varying bioactive potential. Therefore, this study demonstrates the potential of epigenetic modification as an approach for enhancing the production of secondary metabolites from endophytic fungi. Furthermore, it highlights combinatorial strategies as promising approaches for stimulating the antibacterial potential of these fungi in the search for novel antimicrobial candidates.

1. Introduction

Antibiotic resistance has become a global health threat, with an estimated 700,000 deaths per year due to infections caused by resistant bacteria (Mdarrhri et al., 2022). With this estimate expected to continue rising, there is an urgent need for alternative therapeutic strategies to combat antibiotic-resistant infections (Singha et al., 2024). In response, recent studies have explored various potential therapeutic strategies to address this resistance (Aghamohammad and Rohani, 2022). One promising source of novel antimicrobial agents is endophytes, which can produce highly effective bioactive compounds (Nazir et al., 2024). These endophytes, comprising bacteria and fungi, are diverse microorganisms found in healthy plant tissues (Jia et al., 2016). Endophytic fungi alone comprise over one million species in nature and have demonstrated extensive benefits to the host plant and the general ecosystem. The benefits include improving the host's uptake of essential nutrients, protecting the host from phytopathogens, improving tolerance to biotic

and abiotic stressors, and producing secondary metabolites with medicinal and pharmaceutical benefits (Rigobelo and Baron, 2021). Moreover, these fungi produce versatile secondary metabolites with recognized antibacterial and fungicidal activities. Notably, endophytic *Penicillium* has been extensively investigated for its bioactivity, antibiotic potential, and numerous benefits in the biotechnological and pharmaceutical industries (Toghueo and Boyom, 2020). The secondary metabolites produced by endophytic *Penicillium* sp. are mainly bioactive compounds effective as antimicrobial, antioxidant, and anti-inflammatory agents (Weng et al., 2022). Similarly, endophytic fungi in the genus *Aspergillus* are known to produce metabolites with antibacterial, antifungal, anti-inflammatory, and immunosuppressive activities (Hagag et al., 2022). Thus, endophytic fungi are a promising source of novel antimicrobial agents that can reduce reliance on antibiotics.

Despite their potential, several challenges limit the cultivation and study of endophytic fungi. One significant issue is the loss of viability

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and bioactivity when cultured or stored on artificial media. This occurrence has been attributed to their intrinsic location within plant tissues, which significantly limits their ability to thrive under normal laboratory conditions (dos Reis et al., 2022). According to an early study that investigated the nutritional requirements of endophytic fungi, Kulkarni and Nielsen (1986) noted that endophytic fungi used very limited carbon and nitrogen sources for growth. Likewise, a recent study by Akram et al. (2023) found that endophytic fungi do not grow on agar medium. This might be attributed to the rich nutrient content of artificial media, which does not support their metabolism (Akram et al., 2023). Furthermore, dos Reis et al. (2022) noted that growth on culture media requires a specific composition, as varying conditions may inhibit survival. For instance, thiamine was the only vitamin required for the growth of *Ceratophyllum demersum* (Bertoni and Levin, 1997). This further suggests that endophytic fungi thrive under conditions that provide minimal nutrients for their survival (Hume et al., 2013).

In the study by Zakariyah et al. (2024), the authors reported that endophytic fungi often lose their viability when cultured or stored for extended periods. This loss reduces their bioactivity and hinders assessment of their antibiotic potential (Gundel et al., 2010). This occurrence is attributed to the inactivation of biosynthetic gene clusters (BGCs) required to stimulate the production of secondary metabolites (Kjærboelling et al., 2019). Similarly, Shi et al. (2022) emphasized the role of BGCs in mutualistic relationships between organisms, highlighting that BGCs enhance understanding of how microbial natural products interact with their eukaryotic hosts. BGCs are highly specialized genes that regulate the amount and type of bioactive molecules, or secondary metabolites, produced by endophytic fungi (Benite-Ribeiro et al., 2016). Recent advancements in epigenetics have encouraged studies to understand how to prevent BGCs from going silent, reactivate cryptic clusters, and modify gene clusters to enhance the quality and quantity of secondary metabolites. One of the most effective methods of reactivation is epigenetic modification. Epigenetic modification refers to genome alterations that alter a cell's transcriptional potential, leading to changes in gene expression and in cell growth, differentiation, and function (Kumari et al., 2022; Ramesha et al., 2021).

Epigenetic modification of endophytic fungi has been shown to successfully reactivate silent BGCs, leading to the production of new and effective secondary metabolites (Zakariyah et al., 2025). In addition, combining endophytic fungi with conventional antibiotics has a high likelihood of restoring their bioactivity (Omokhua-Uyi et al., 2025). This way, antibiotics act as an adjuvant, broadening the spectrum of activity of endophytic fungi through a synergistic mechanism (Moussa, 2024). In turn, this preserves the efficacy of antibiotics, reduces dependence on them as sole antimicrobial agents, and reduces antimicrobial resistance. However, only a few studies have examined the antibacterial potential of metabolites from epigenetically modified endophytic fungi and the combined effect of antibiotics on endophytic fungal activity. Therefore, this study aims to investigate the viability of endophytic fungi grown and stored on culture media. It also provides insights into the antibacterial potential of secondary metabolites from epigenetically modified endophytic *Penicillium* and *Aspergillus* spp., and into the synergistic effect of antibiotics in restoring the viability and antibacterial potential of the selected endophytic fungi.

2. Methodology

2.1. Cultivation and viability of the selected endophytic fungi

The selected endophytic fungi, *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6, were obtained from Zakariyah et al. (2025). The endophytic fungi were successively subcultured into three generations on Potato Dextrose Agar to determine their viability. Viability was determined by measuring mycelial growth diameter (MGD) every 48 h across the three generations. The equation for calculating the MGD was obtained from the work of Werghemmi et al.

(2022):

$$\text{Mycelial Growth Diameter (mm)} = \frac{D1 + D2}{2} \quad (1)$$

2.2. Source of the test organisms

The test organisms used for this study, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Acinetobacter* spp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, were collected from the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital, University of Ilorin.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the extract was performed by slightly modifying the method described by Ali et al. (2022). The test organisms were inoculated onto sterile Mueller-Hinton agar plates using sterile swab sticks, and the plates were labeled with the corresponding organisms. The antimicrobial activity of the endophytic fungi and their extracts was tested using agar plug and microbroth dilution methods, respectively.

2.3.1. Agar plug method for the primary screening of the selected endophytic fungi

Four (4) equidistant holes were made on each agar plate using a 7 mm sterile cork borer. Actively growing endophytic fungi were introduced into these holes, and Gentamicin served as a positive control. Plates were incubated at 37 °C for 18–24 h. Antimicrobial activity was evaluated by measuring the clear inhibition zones around the holes to the nearest millimeter using a ruler.

2.3.2. Microbroth dilution method for the secondary screening of the selected endophytic fungi

About 50 µL of Mueller-Hinton Broth (MHB) was added to the microplates. The extracts were reconstituted to 512 µg/mL, and 50 µL of the solution was added to the first well. Serial two-fold dilutions were then carried out by transferring 50 µL from one well to the next along the row, mixing with 50 µL of MHB in each subsequent well, to obtain a dilution series. After this, 10 µL of the standardized test organisms was added. The plates were then incubated at 37 °C for 24 h. A colorimetric indicator (INT: p-iodonitrotetrazolium violet) was added to the wells to characterize microbial growth, and the microplates were re-incubated for 30 min.

2.4. Determination of the minimum inhibitory concentration (MIC)

A broth microdilution susceptibility assay was used to determine the MIC. All tests were performed using Muller-Hinton broth and 96-well Microplates. 70 µL of MHB was added to the microplates, and the extracts were reconstituted to 512 µg/mL and diluted in serial steps. Then, 10 µL of the standardized test organisms was added. The plates were then incubated at 37 °C for 24 h. INT was then added to the wells to characterize microbial growth, and the microplates were re-incubated for 30 min.

2.5. Determination of the minimum bactericidal concentration (MBC)

The MBC was determined by plating out the MIC. Sterile nutrient agar was poured into the plates and incubated. The MBC endpoint was the lowest concentration and killed ≥ 99.9% of the test bacteria.

2.6. Reactivation of the viability and antibacterial potential using epigenetic modification

and *Penicillium citrinum* isolate UILRZ6, the fermentation medium

was modified with epigenetic modifiers, as described by Makhwitine et al. (2023). Four epigenetic modifiers were used to broaden the reactivation of silent BGCs and enhance secondary metabolite production by endophytic fungi. The fungi were pre-incubated on Potato Dextrose Broth at 27 ± 2 °C for 7 days. A one-liter sterile liquid fermentation broth was prepared by mixing 60 g of cornmeal, 20 g of sucrose, 3 g of yeast extract, 0.25 g of MgSO_4 , 5 g of KH_2PO_4 , 0.1 g of MgCl_2 , 0.01 g of ZnSO_4 , and 0.01 g of FeSO_4 . The epigenetic modifiers were dissolved in distilled water and aseptically poured into each flask to achieve a final concentration of 500 μM . The broth was mixed thoroughly and adjusted to a pH of 5.0. A sterile cork borer was used to transfer the actively growing endophytic fungi isolates (six inoculum plugs) into each conical flask containing the sterile broth under aseptic conditions. The flasks were incubated at 28 ± 2 °C and 120 rpm for 7 days. Ethanol was added to the filtrate in a 3:1 ratio for maximal extraction. The filtrate collected was evaporated using a rotary evaporator and lyophilized using a Labconco freeze dryer. The antibacterial screening, MIC, and MBC were evaluated as previously described. *P. aeruginosa* was selected as the sole test organism at this stage based on its resistance profile as a WHO critical-priority pathogen, high clinical relevance, and global burden.

2.7. Combination therapy with penicillin

The extracts of the epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 were combined with penicillin in a ratio of 1:1. This was done to assess the influence of penicillin on the antibacterial activity of the endophytic fungi. Antibacterial screening, MIC, and MBC against *P. aeruginosa* were evaluated as previously described.

2.8. Gas chromatography-mass spectrometry (GC-MS) analysis

The non-polar chemical composition of the endophytic fungi was subjected to GC-MS analysis, as previously reported. A capillary column HP-5 (30 m x 0.25 mm internal diameter; CM Scientific, USA), coated with a 0.25 μm film of 5% (v/v) dimethyl polysiloxane and 95% diphenyl, was used for separation. The column temperature was set at 100 °C and held for 5 min. A 0.5 μL sample solution (5 mg of petroleum ether extracts dissolved in 1 mL of PE, while dichloromethane extracts were dissolved in 1 mL of CH_2Cl_2) was injected. The initial column temperature was then increased to 145 °C at a rate of 5 °C/min and held at this temperature for 25 min. The MS inlet temperature was 250 °C; MS mode: (EI, mass range, 40–550 amu ionization source temperature 280 °C). The compounds were identified based on retention indices (RI) and by comparing the spectra with a stored MS library (W8N05ST and NIST08).

2.9. Statistical analysis

The statistical analysis of the antibacterial activity of the endophytic fungi was conducted using SPSS version 20, with the zone of inhibition as the dependent variable. The data were analyzed using one-way Analysis of Variance (ANOVA) and expressed as mean $\pm$ standard deviation. Pearson correlations were also applied to assess the relationships among the test groups. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Viability of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The viability of the endophytic fungi decreased progressively as the number of subcultures increased. This decrease is particularly evident in the *Aspergillus* sp. isolate UILRZ5, which lost viability by the third

generation. The result is calculated as the mycelial growth rate across the three generations, shown in Table 1.

3.2. Antibacterial activity of the *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The antibacterial activity of the three generations of endophytic fungi was determined against six test organisms. The result of the susceptibility testing is presented in Table 2. *Penicillium citrinum* isolate UILRZ6 had better activity against the test organisms than *Aspergillus* sp. isolate UILRZ5, except for *Klebsiella pneumoniae*. *K. pneumoniae* was resistant to the two endophytic fungi, as shown by the lack of zones of inhibition. In contrast, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were highly susceptible to the endophytic fungi.

3.3. Minimum inhibitory concentration of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

Table 3 presents the MIC of extracts from the selected endophytic fungi *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6. Both endophytic fungi showed strong bacteriostatic activity against *Staphylococcus aureus*, with only 32 $\mu\text{g/mL}$ of each extract required to inhibit its growth. However, the reverse is true for *K. pneumoniae*, which requires 256 $\mu\text{g/mL}$ of both extracts.

3.4. Minimum bactericidal concentration of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The bactericidal effects of the endophytic fungal isolates against the test organisms are presented in Table 3. The MBC of both extracts ranged from 32 to 512 $\mu\text{g/mL}$. *Penicillium citrinum* isolate UILRZ6 exhibited a better bactericidal effect on all the test organisms than *Aspergillus* sp. isolate UILRZ5. In addition, the highest concentration of *Aspergillus* sp. isolate UILRZ5 extract was ineffective against *Pseudomonas aeruginosa*.

3.5. Antibacterial activity of the epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The antimicrobial activity of the epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 was determined against seven strains of *P. aeruginosa*. Based on the result in Table 4, extracts of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 modified with Quercetin were ineffective against all seven strains of *P. aeruginosa*. In contrast, the test organisms reacted more favorably to the *Penicillium citrinum* isolate UILRZ6 modified with nicotinamide than to the other extracts tested.

3.6. Minimum inhibitory concentration of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The MIC of the extracts from epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 was tested against *P. aeruginosa*. The MICs of all the modified extracts ranged from 32 $\mu\text{g/mL}$ to 256 $\mu\text{g/mL}$. Although *Penicillium citrinum* isolate UILRZ6 modified with nicotinamide inhibited all the strains of the test organisms, lower concentrations of *Aspergillus* sp. isolate UILRZ5 modified with

Table 1
Viability of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6.

Endophytic Fungi	1 st Generation (mm)	2 nd Generation (mm)	3 rd Generation (mm)
<i>Aspergillus</i> sp. isolate UILRZ5	85.0	59.5	0.00
<i>P. citrinum</i> isolate UILRZ6	75.0	67.5	32.5

Table 2Antimicrobial Activity of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6.

Zone of Inhibition of Test Organisms (mm)							
TA	Generation	EC	PA	KP	SA	AB	EF
A	First	7.75 ± 0.25	11.50 ± 1.53	0.00	12.00 ± 0.00	0.00	0.00
P	First	10.00 ± 0.00	8.25 ± 0.75	0.00	13.50 ± 0.50	7.00 ± 0.00	0.00
A	Second/Third	0.00	0.00	0.00	0.00	0.00	0.00
P	Second/Third	0.00	0.00	0.00	0.00	0.00	0.00
Gentamicin	0.00	22.0 ± 0.82	0.00	24.0 ± 1.50	11.0 ± 0.82	16.0 ± 1.63	

The test was done in triplicate (n = 3). Values are given as Mean ± Standard Deviation at a significance level of 95% according to the ANOVA test (P < 0.05). Gentamicin was used as a positive control.

KEY

TA:Test Agent

EC:Escherichia coliEF:Enterococcus faecalis

PA:Pseudomonas aeruginosaAB:Acinetobacter spp.

KP:Klebsiella pneumoniaeSA:Staphylococcus aureus

A:Aspergillus sp. isolate UILRZ5P:Penicillium citrinum isolate UILRZ6

Table 3MIC and MBC of extracts of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6.

Test Organism	MIC (µg/mL)		MBC (µg/mL)	
	A	P	A	P
<i>Escherichia coli</i>	256	32	256	128
<i>Pseudomonas aeruginosa</i>	256	128	NA	256
<i>Klebsiella pneumoniae</i>	256	256	512	256
<i>Staphylococcus aureus</i>	32	32	32	64
<i>Acinetobacter baumannii</i>	128	128	256	128
<i>Enterococcus faecalis</i>	64	32	256	128

KEY

A:Aspergillus sp. isolate UILRZ5P:Penicillium citrinum isolate UILRZ6

NA:No Activity

hydralazine hydrochloride were required to inhibit the organisms (32 µg/mL for three strains of *P. aeruginosa*). The result is shown in Table 5.

3.7. Minimum bactericidal concentration of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

The minimum bactericidal effect of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 against *P. aeruginosa* is presented in Table 5. From the result, the MBC required to kill a susceptible strain of test organism was 128 µg/mL, then 256 µg/mL. In addition, one strain of the test organism (*P. aeruginosa* 100)

resisted all three epigenetically modified extracts. Although most of the test strains were resistant, the endophytic fungi extract modified with nicotinamide demonstrated better bactericidal activity against the susceptible strains of *P. aeruginosa*.

3.8. Antibacterial activity of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 combined with penicillin

The epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 were combined with penicillin and screened for antibacterial activity against *P. aeruginosa*. Nicotinamide-modified *Penicillium citrinum* isolate UILRZ6 was the most effective extract, with activity ranging from 3.7 ± 0.32–12.1 ± 0.15. Hydralazine and nicotinamide-modified *Aspergillus* sp. isolate UILRZ5 with penicillin were significantly effective against *P. aeruginosa* strain 914, with the highest zones of inhibition of 19.23 ± 0.25 and 14.13 ± 0.23, respectively. The result is presented in Table 6.

3.9. Minimum inhibitory concentration of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 combined with penicillin

The extracts of the epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 were combined with penicillin. The resulting extracts were assayed for their MIC against *P. aeruginosa* and presented in Table 7. Although *Penicillium citrinum* isolate UILRZ6 with nicotinamide and penicillin possessed inhibitory effects against all the strains of *P. aeruginosa*, *Aspergillus* sp. isolate

Table 4Antibacterial activity of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6.

Test	P HD	P SB	P NIC	P Q	A HD	A SB	A NIC	A Q
Org.PA	0.00	0.00	0.00	0.00	0.00	9.20 ± 0.30	6.37 ± 0.15	0.00
PA 70	0.00	0.00	4.26 ± 0.25	0.00	4.73 ± 0.21	6.77 ± 0.25	6.57 ± 0.40	0.00
PA 80	0.00	0.00	4.76 ± 0.15	0.00	5.27 ± 0.25	6.47 ± 0.25	6.37 ± 0.15	0.00
PA 90	0.00	0.00	7.46 ± 0.50	0.00	0.00	0.00	0.00	0.00
PA 100	0.00	0.00	3.70 ± 0.25	0.00	0.00	0.00	0.00	0.00
PA 913	0.00	0.00	4.86 ± 0.25	0.00	3.20 ± 0.26	6.23 ± 0.25	5.03 ± 0.15	0.00
PA 914	0.00	0.00	2.67 ± 0.15	0.00	9.33 ± 0.21	5.00 ± 0.20	7.27 ± 0.25	0.00

The test was done in triplicate (n = 3). Values are given as Mean ± Standard Deviation at a significance level of 95% according to the ANOVA test (P ≤ 0.05).

KEY

A HD: *Aspergillus* sp. isolate UILRZ5 + Hydralazine hydrochloride PA: *Pseudomonas aeruginosa*A SB: *Aspergillus* sp. isolate UILRZ5 + Sodium butyrateA NIC: *Aspergillus* sp. isolate UILRZ5 + NicotinamideA Q: *Aspergillus* sp. + QuercetinP NIC: *Penicillium citrinum* isolate UILRZ6 + NicotinamideP Q: *Penicillium citrinum* isolate UILRZ6 + QuercetinP SB: *Penicillium citrinum* isolate UILRZ6 + Sodium butyrateP HD: *Penicillium citrinum* isolate UILRZ6 + Hydralazine hydrochloride

Table 5

MIC and MBC of Epigenetically Modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6.

Test Org.	MIC (µg/mL)				MBC (µg/mL)			
	P NIC	AN HD	AN SB	AN NIC	P NIC	AN HD	AN SB	AN NIC
PA	NA	32	256	256	NA	NA	128	256
PA 70	128	256	256	256	NA	NA	128	256
PA 80	128	32	32	32	NA	NA	NA	NA
PA 90	64	NA	NA	NA	256	NA	NA	NA
PA 100	64	NA	NA	NA	NA	NA	NA	NA
PA 913	128	32	64	256	128	NA	NA	NA
PA 914	128	128	NA	128	256	NA	NA	128

The MIC and MBC for A Q, P Q, P SB and P HD were not evaluated as they had no antibacterial activity and were not included in subsequent analyses.

KEY

A HD: *Aspergillus* sp. isolate UILRZ5 + Hydralazine hydrochloride **PA:** *Pseudomonas aeruginosa*

A SB: *Aspergillus* sp. isolate UILRZ5 + Sodium butyrate

A NIC: *Aspergillus* sp. isolate UILRZ5 + Nicotinamide

A Q: *Aspergillus* sp. + Quercetin

P Q: *Penicillium citrinum* isolate UILRZ6 + Quercetin

P NIC: *Penicillium citrinum* isolate UILRZ6 + Nicotinamide

P SB: *Penicillium citrinum* isolate UILRZ6 + Sodium butyrate

P HD: *Penicillium citrinum* isolate UILRZ6 + Hydralazine hydrochloride

Table 6

Antibacterial activity of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 combined with penicillin.

TO	P NIC + Pen	A HD + Pen	A SB + Pen	A NIC + Pen
PA	0.00	2.20 ± 0.20	15.10 ± 0.26	11.40 ± 0.20
PA 70	10.5 ± 0.47	9.23 ± 0.25	11.20 ± 0.26	7.27 ± 0.25
PA 80	8.4 ± 0.46	9.47 ± 0.25	13.17 ± 0.21	9.53 ± 0.35
PA 90	8.40 ± 0.17	0.00	0.00	0.00
PA 100	12.1 ± 0.15	0.00	0.00	0.00
PA 913	3.7 ± 0.32	8.27 ± 0.21	10.23 ± 0.25	7.30 ± 0.20
PA 914	6.72 ± 0.36	19.23 ± 0.25	8.27 ± 0.25	14.13 ± 0.23

The test was done in triplicate (n = 3). Values are given as Mean ± Standard Deviation at a significance level of 95% according to the ANOVA test (P ≤ 0.05).

KEY

A HD + Pen: *Aspergillus* sp. isolate UILRZ5 + Hydralazine hydrochloride + Penicillin

A SB + Pen: *Aspergillus* sp. isolate UILRZ5 + Sodium butyrate + Penicillin

A NIC + Pen: *Aspergillus* sp. isolate UILRZ5 + Nicotinamide + Penicillin

P NIC + Pen: *Penicillium citrinum* isolate UILRZ6 + Nicotinamide + Penicillin

UILRZ5 with sodium butyrate and penicillin exhibited better inhibitory effects. This is because only 32 µg/mL of the extract was required to inhibit four strains of the test organism. Additionally, *P. aeruginosa* 90

Table 7

MIC and MBC of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 combined with penicillin.

Test Org.	MIC (µg/mL)				MBC (µg/mL)			
	P NIC + Pen	A HD + Pen	A SB + Pen	A NIC + Pen	P NIC + Pen	A HD + Pen	A SB + Pen	A NIC + Pen
PA	NA	32	32	128	NA	NA	NA	NA
PA 70	256	128	128	128	NA	NA	64	NA
PA 80	128	32	32	32	NA	NA	NA	NA
PA 90	128	NA	NA	NA	256	NA	NA	NA
PA 100	256	NA	NA	NA	256	NA	NA	NA
PA 913	32	16	32	64	NA	NA	NA	NA
PA 914	64	64	32	16	NA	NA	NA	NA

KEY

A HD + Pen: *Aspergillus* sp. isolate UILRZ5 + Hydralazine hydrochloride + Penicillin

A SB + Pen: *Aspergillus* sp. isolate UILRZ5 + Sodium butyrate + Penicillin

A NIC + Pen: *Aspergillus* sp. isolate UILRZ5 + Nicotinamide + Penicillin

P NIC + Pen: *Penicillium citrinum* isolate UILRZ6 + Nicotinamide + Penicillin

PA: *Pseudomonas aeruginosa*

and 100 resisted all the extracts of *Aspergillus* sp. isolate UILRZ5 combined with penicillin.

3.10. Minimum bactericidal concentration of epigenetically modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 combined with penicillin

The Epigenetically modified extracts of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 were combined with penicillin and screened for their MBC against *P. aeruginosa*. Based on the results in Table 7, all the strains of *P. aeruginosa* resisted the combination of *Aspergillus* sp. isolate UILRZ5 with nicotinamide and penicillin. Only one strain (*P. aeruginosa* 70) was susceptible to the combination of *Aspergillus* sp. isolate UILRZ5 with sodium butyrate and penicillin (64 µg/mL). At the same time, two (*P. aeruginosa* 90 and 100) were susceptible to Nicotinamide-modified *Penicillium citrinum* isolate UILRZ6 combined with penicillin. Furthermore, the minimum concentration of the combined therapy required to kill these two was 256 µg/mL.

3.11. Descriptive statistics

Table 8 below shows the descriptive statistics of the MIC of the endophytic fungi before epigenetic modification (A, P), after epigenetic modification (NIC, HD, SB) and the combination therapy with penicillin. Quercetin was not included as it was largely ineffective throughout the investigation. The mean MIC values vary across the three treatment sets, indicating differences in their antibacterial activity. *Aspergillus* sp. isolate UILRZ5 had the highest mean at 165.33 ± 104.021 , suggesting that a very high concentration is required to inhibit bacterial growth. On the other hand, the extracts modified with sodium butyrate and hydralazine hydrochloride combined with penicillin had the lowest mean values at 36.29 ± 43.331 and 38.57 ± 45.409 , respectively.

3.12. Inferential statistics

I) Analysis of variance (ANOVA)

The table below (Table 9) shows a p-value greater than 0.05, indicating that the differences in the endophytic fungi MIC values are not statistically significant. This means that the antibacterial effectiveness of the endophytic fungi extracts is similar. Hence, all the extracts and treatment sets have a similar inhibitory effect on the test organisms.

I) Levene's Test

The Levene test determines the equality of variances across two or more sample groups. The table below shows the results of Levene's test conducted for the endophytic fungi MIC sets. The p-value is statistically

Table 8

Descriptive statistics of the minimum inhibitory concentration of endophytic fungal extracts.

Extract Treatments	Mean $\pm$ SD ($\mu\text{g/mL}$)	Standard Error	95% Confidence Interval		Minimum	Maximum
			Lower	Upper		
NIC	91.29 $\pm$ 50.658	19.147	44.43	138.14	-1	128
HD	68.29 $\pm$ 93.411	35.306	-18.10	154.68	-1	256
SB	86.43 $\pm$ 118.255	44.696	-22.94	195.80	-1	256
NIC + Pen	123.29 $\pm$ 102.115	38.596	28.84	217.73	-1	256
HD + Pen	38.57 $\pm$ 45.409	17.163	-3.42	80.57	-1	128
SB + Pen	36.29 $\pm$ 43.331	16.378	-3.79	76.36	-1	128
A	165.33 $\pm$ 104.021	42.466	56.17	274.50	32	256
P	101.33 $\pm$ 89.180	36.408	7.74	194.92	32	256

KEY**A:** *Aspergillus* sp. isolate UILRZ5 **HD:** Hydralazine hydrochloride**P:** *Penicillium citrinum* isolate UILRZ6 **+Pen:** Combined with Penicillin**SB:** Sodium butyrate**NIC:** Nicotinamide**Table 9**

ANOVA of the Minimum Inhibitory Concentration (MIC) of endophytic fungal extracts.

Source of Variations	Sum of Squares	df	Mean Square	F	p-value
Between Groups	106954.083	9	11883.787	1.559	0.150
Within Groups	442256.667	58	7625.115		
Total	549210.750	67			

Table 10

Homogeneity of variance (Levene Test) of the Minimum Inhibitory Concentration (MIC) of endophytic fungal extracts.

Parameter	Levene Statistic	df	Df2	p-value
MIC	3.065	9	58	0.005

Table 11

Analysis of the minimum inhibitory concentration using the Kruskal-Wallis test.

Extract Treatments	N (Sample size)	Mean Rank of MIC
A	6	50.08
NIC + Pen	7	42.14
NIC	7	40.00
P	6	39.75
SB	7	30.43
HD	7	29.36
HD + Pen	7	24.50
SB + Pen	7	24.14

KEY**A:** *Aspergillus* sp. isolate UILRZ5 **HD:** Hydralazine hydrochloride**P:** *Penicillium citrinum* isolate UILRZ6 **+Pen:** Combined with Penicillin**SB:** Sodium butyrate**NIC:** Nicotinamide**I) Correlation analysis**

significant ($p < 0.05$), suggesting that the variation in MICs is unequal. Therefore, the MIC values vary differently and significantly across the extract treatment sets.

I) Kruskal-Wallis Test (Non-Parametric Test)

Since the ANOVA was not statistically significant and Levene's test was significant, the Kruskal-Wallis test was conducted as a confirmatory test. The Kruskal-Wallis test was used to determine if independent samples originated from the same distribution. The table below shows the test results for the MIC sets ranked by their effectiveness against the test organisms. The extracts at the higher end of the table (*Aspergillus* sp. isolate UILRZ5 and nicotinamide combined with penicillin) have higher

mean MIC ranks, indicating lower effectiveness and antibacterial potential. In contrast, sodium butyrate and hydralazine hydrochloride combined with penicillin have lower MIC values and rank lower, indicating their greater antibacterial potential. However, the p-value is greater than 0.05 (0.211), indicating no significant difference between the MICs across the extract treatment sets. Therefore, despite the ranking, the test did not ascertain that the differences in antibacterial potential were statistically significant.

The correlation analysis in Table 12 shows the relationships among the MIC values obtained from the three extract treatments. The table shows a weak, positive correlation between the MIC values of the endophytic fungi and those of the epigenetically modified extracts ($r = 0.151$, $p = 0.576$). A similar relationship was observed between the MICs of the epigenetically modified extracts alone and when combined with penicillin ($r = 0.116$, $p = 0.591$). This may mean that an increase in MIC values in one treatment is associated with a similar increase in the other. In contrast, there is a weak negative correlation between the endophytic fungi extracts and the epigenetically modified extracts combined with penicillin ($r = -0.029$, $p = 0.916$). This may mean that a decrease in one leads to an increase in the other. However, not all relationships were statistically significant, showing that they may be due to chance. Also, it indicates that modifying endophytic fungi and combining them with penicillin does not consistently or predictably affect MIC values. Therefore, the antibacterial potential of the treatment options is independent of each other.

3.13. Chemical compounds detected from the extracts of *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

Gas Chromatography-Mass Spectrometry (GC-MS) was utilized to analyze the constituents of the extracts of the endophytic *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6. The results of the analyses are presented in Tables 13 and 14. For the *Aspergillus* sp. isolate UILRZ5, the molecular weight of the compounds in its extract ranges from 222 g/mol to 294 g/mol. Diethyl benzene-1,2-dicarboxylate had the lowest amount, at 4.02%, while Oleic acid had the highest, at 45.79%. The molecular weights of the chemical compounds found in *Penicillium citrinum* isolate UILRZ6 extracts range from 77 g/mol to

Table 12

Correlation matrix of the MIC values of the endophytic fungi.

MIC values and their correlations		MIC	MIC (EM)	MIC (EM+P)
MIC	Pearson Correlation (r)	1	0.151	-0.029
	p-value		0.576	0.916
MIC (EM)	Pearson Correlation (r)	0.151	1	0.116
	p-value	0.576		0.591
MIC3 (EM+P)	Pearson Correlation (r)	-0.029	0.116	1
	p-value	0.916	0.591	

Table 13Chemical constituents of the *Aspergillus* sp. isolate UILRZ5 Extract.

Peaks	Retention Time (secs)	Compounds	Area (%)	Molecular Formula	Weight (g/mol)
1	36.375	Methyl hexadecanoate	25.26	C ₁₇ H ₃₄ O ₂	270
2	41.355	methyl (10 <i>E</i> ,12 <i>Z</i>)-octadeca-10,12-dienoate	13.60	C ₁₉ H ₃₄ O ₂	294
3	41.587	Oleic acid	45.79	C ₁₈ H ₃₄ O ₂	282
4	42.475	Methyl hexadecanoate	11.33	C ₁₇ H ₃₄ O ₂	270
5	53.509	2-butoxycarbonylbenzoic acid	4.02	C ₁₂ H ₁₄ O ₄	222

Table 14Chemical constituents of the *Penicillium citrinum* isolate UILRZ6 extract.

Peaks	Retention Time (min)	Compounds	Area (%)	Molecular Formula	Weight (g/mol)
1	5.681	<i>N</i> -methoxy- <i>N</i> -methylhydroxylamine	5.88	C ₂ H ₇ NO ₂	77
2	8.940	2-butoxyethanol	1.27	C ₆ H ₁₄ O ₂	118
3	9.716	Ethyl prop-2-ynoate	39.50	C ₅ H ₆ O ₂	98
4	12.656	Cyclopentane-1,2-dione	4.99	C ₇ H ₁₀ O ₃	142
5	16.334	Maltol	1.29	C ₆ H ₆ O ₃	126
6	18.561	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.45	C ₆ H ₈ O ₄	144
7	29.818	5-Ethyl-1 <i>H</i> -1,2,4-triazole	1.96	C ₄ H ₇ N ₃	97
8	28.576	9-Amino-7-mercapto-5,6,8,10-tetraaza-benzo[<i>b</i>]fluoren-11-one	1.32	C ₁₃ H ₇ N ₃ OS	281
9	30.046	3,4-Altrosan	1.40	C ₆ H ₁₀ O ₅	162
10	31.034	2,4- <i>ditert</i> -butylphenol	1.22	C ₁₄ H ₂₂ O	206
11	37.252	2,3-diphenyl-1 <i>H</i> -1,2,4-triazole-5-thione	1.31	C ₁₄ H ₁₁ N ₃ S	253
12	38.753	Dipentyl benzene-1,2-dicarboxylate	3.97	C ₁₈ H ₂₈ O ₄	306

306 g/mol. Cyclopentane-1,2-dione was the most abundant compound in the extract (39.50%), while 2,4-*ditert*-butylphenol was the least (0.14%).

Table 15Chemical constituents of sodium butyrate-modified *Aspergillus* sp. isolate UILRZ5 extract.

Peaks	Retention time (Secs)	Compounds	Area (%)	Formula	Weight (g/mol)
1	6.669	Butane-1,2,3-triol	0.83	C ₄ H ₁₀ O ₃	106
2	6.859	Pantolactone	0.33	C ₆ H ₁₀ O ₃	130
3	7.410	Methyl furan-2-carboxylate	0.23	C ₆ H ₆ O ₃	126
4	7.522	1-Nitrosopiperazine	0.19	C ₄ H ₉ N ₃ O	115
5	8.074	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.28	C ₆ H ₈ O ₄	144
6	8.335	2,3-Anhydro- <i>D</i> -mannosan	0.28	C ₆ H ₈ O ₄	144
7	8.529	Mannitan	0.48	C ₆ H ₁₂ O ₅	164
8	8.585	1,4:3,6-Dianhydro- α - <i>D</i> -glucopyranose	0.52	C ₆ H ₈ O ₄	144
9	8.648	Dianhydromannitol	5.06	C ₆ H ₁₀ O ₄	146
10	8.783	Ethyl decanoate	0.28	C ₁₂ H ₂₄ O ₂	198
11	9.020	Niacin	0.51	C ₆ H ₅ NO ₂	123
12	9.265	3-hydroxyoctanoic acid	0.23	C ₈ H ₁₆ O ₃	160
13	9.367	(<i>Z</i>)-3-Methyl-2-hexenoic acid	1.29	C ₇ H ₁₂ O ₂	128
14	11.321	Betonidine	0.66	C ₇ H ₁₃ NO ₃	159
15	12.865	Diethyl benzene-1,2-dicarboxylate	3.49	C ₁₂ H ₁₄ O ₄	222
16	14.885	Tridecanoic acid	0.19	C ₁₃ H ₂₆ O ₂	214
17	16.601	<i>Cis</i> -10-Nonadecenoic acid	0.27	C ₁₉ H ₃₆ O ₂	296
18	16.666	Diethyl benzene-1,2-dicarboxylate	0.57	C ₁₆ H ₂₂ O ₄	278
19	16.803	Hexadecanoic acid	13.67	C ₁₆ H ₃₂ O ₂	256
20	17.129	Ethyl palmitate	7.04	C ₁₈ H ₃₆ O ₂	284
21	17.522	Oleyl alcohol, trifluoroacetate	0.23	C ₂₀ H ₃₅ F ₃ O ₂	364
22	18.051	8,11,14-Eicosatrienoic acid-(<i>Z,Z,Z</i>)	0.25	C ₂₀ H ₃₄ O ₂	306
23	18.157	(9 <i>Z</i> ,12 <i>Z</i>)-octadeca-9,12-dienoic acid	2.62	C ₁₈ H ₃₂ O ₂	280
24	18.216	Oleic acid	11.01	C ₁₈ H ₃₄ O ₂	282
25	18.403	Octadecanoic acid	16.28	C ₁₈ H ₃₆ O ₂	284
26	18.473	Ethyl oleate	9.76	C ₂₀ H ₃₈ O ₂	310
27	18.668	Ethyl octadecanoate	11.38	C ₂₀ H ₄₀ O ₂	312
28	19.775	20,20,20-trideuterioicosanoic acid	0.34	C ₂₀ H ₄₀ O ₂	312
29	20.014	Ethyl hexadecanoate	0.94	C ₁₈ H ₃₆ O ₂	284
30	20.684	3,3-dihydroxypropyl hexadecanoate	0.28	C ₁₉ H ₃₈ O ₄	330
31	20.915	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	0.32	C ₂₄ H ₃₈ O ₄	390
32	20.957	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	0.52	C ₂₄ H ₃₈ O ₄	390
33	21.426	Ethyl nonadecanoate	0.59	C ₂₁ H ₄₂ O ₂	326
34	22.042	2,3-dihydroxypropyl (<i>Z</i>)-octadec-9-enoate	7.24	C ₂₁ H ₄₀ O ₄	356
35	23.139	Ethyl tetracosanoate	0.60	C ₂₆ H ₅₂ O ₂	396
36	23.785	Trimethyl-(2-trimethylsilylphenyl)silane	0.24	C ₁₂ H ₂₂ Si ₂	222
			100.00		

3.14. Chemical compounds detected from the extracts of epigenetically Modified *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6

GC-MS was used to analyze the constituents of the extract of *Aspergillus* sp. isolate UILRZ5 epigenetically modified with sodium butyrate and *Penicillium citrinum* isolate UILRZ6 epigenetically modified with nicotinamide. The results of the analyses are presented in Tables 15 and 16. For *Aspergillus* sp. isolate UILRZ5, the weight of the compounds in its extract ranges between 106 g/mol and 396 g/mol. Tridecanoic acid and 1-Nitrosopiperazine had the lowest quantities, with a percentage of 0.19%, while Octadecanoic acid had the highest at 16.28%. The weight of the chemical compounds found in *Penicillium citrinum* isolate UILRZ6 extracts ranges from 106 g/mol to 390 g/mol. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was the most abundant compound in the extract (12.16%), while Methyl (Z)-octadec-9-enoate was the least (0.14%).

4. Discussion

The bioactivity of endophytic fungi makes them suitable candidates for the search for novel therapeutic compounds to combat antimicrobial resistance. However, some studies have shown that they are fragile and prone to loss of viability and bioactivity following extended storage (Akram et al., 2023; Hume et al., 2013). Building on previous findings that highlighted the loss of viability and antibacterial potential in *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 (Zakariyah et al., 2025), this study explored epigenetic modification and antibiotic combination therapy as suitable strategies to reverse this loss. To our knowledge, this study is among the first to evaluate the use of these techniques to restore and enhance the viability and antibacterial activity of endophytic fungi following subculturing.

The initial observation revealed that the *Aspergillus* sp. isolate UILRZ5 lost viability by the third generation, whereas the *Penicillium citrinum* isolate UILRZ6 remained viable but showed poor growth. This finding may be due to endophytic fungi thriving within host plants and requiring environments similar to those of their hosts to survive (Vázquez-de-Aldana et al., 2019). This contrasts with the results of Ngoc and Thanh (2022), who reported that endophytic fungi are generally easy to cultivate under regulated conditions. Additionally, endophytic fungi require minute amounts of nutrients, specific growth factors, and culture conditions for effective growth (dos Reis et al., 2022). Thus, nutrient limitations, slow growth rate, and production of toxic metabolites may have negatively impacted their growth and survival.

Despite these challenges in maintaining viability, the endophytic fungi were highly potent against *S. aureus*, *P. aeruginosa*, and *E. coli*. The complete lack of inhibition zones for *K. pneumoniae* demonstrates the extent of its resistance. Abdelgawad et al. (2022) reported similar findings, highlighting that *Aspergillus* sp. exhibits strong antibacterial activity against *S. aureus* and *E. coli* but not against *K. pneumoniae*. Although our antibacterial assay indicated moderate susceptibility of *P. aeruginosa*, other studies have highlighted its resistance. One such study by Abdelgawad et al. (2022) demonstrated that under the activity of *Aspergillus* sp., *P. aeruginosa* had an inhibition percentage of 30.25%. A notable discrepancy was observed between the primary screening and subsequent antibacterial assays. While the MIC and MBC of both endophytic fungi showed inhibitory activity against *K. pneumoniae* and *E. faecalis*, no zones of inhibition were observed during the primary screening. This discrepancy may be attributed to methodological differences between the assays. The agar plug diffusion technique depends on the solubility and diffusion of compounds into the surrounding agar (Scorzoni et al., 2016), which may have limited the quality and concentration of the secondary metabolites secreted and affected their activity. In contrast, the microbroth dilution assay allowed the use of fungal extracts containing a uniform concentration of secondary metabolites, thereby enhancing the activity observed in the MIC and MBC.

Similar observations have been reported by previous studies. For instance, Mamangkey et al. (2024) noted that the renewed susceptibility of organisms in broth assays is attributed to the presence and concentration of potent bioactive compounds, such as penochrochlacones and butyrolactones, in the fungal extracts.

While the initial screenings demonstrated variability in bacterial susceptibility, the MIC results showed that *P. aeruginosa* was highly resistant, requiring 256 µg/mL of *Aspergillus* sp. isolate UILRZ5 and 128 µg/mL of *Penicillium citrinum* isolate UILRZ6 extracts to be inhibited. Furthermore, the *Aspergillus* extract was ineffective in mounting a bactericidal effect on the bacterium. *P. aeruginosa* has a high clinical prevalence, especially in hospital-acquired infections. It is frequently isolated from patients with ventilator-associated pneumonia, urinary tract infections, and bloodstream infections, and is notably more common than many other Gram-negative pathogens in intensive care units (Weiner-Lastinger et al., 2020). Additionally, the classification of *P. aeruginosa* as a WHO critical-priority pathogen, coupled with its adaptive resistance mechanisms and global burden (Elfadadny et al., 2024; Wester et al., 2024), made it the most appropriate sole target for subsequent screening stages.

This led us to explore epigenetic modification as a strategy to reactivate the viability and reverse the antibacterial potential of the selected endophytic fungi, specifically targeting *P. aeruginosa*. The findings demonstrate that the modifiers were more effective in reactivating the bioactivity of the *Aspergillus* sp. isolate UILRZ5, whereas sodium butyrate and hydralazine hydroxide were ineffective for the *Penicillium citrinum* isolate UILRZ6. These findings may be attributed to differences in the uptake of the modifiers based on the growth rate and metabolism of both endophytic fungi (Matús et al., 2023). Quercetin was ineffective, as evidenced by the lack of activity of both modified endophytic fungi against the test strains. In contrast, nicotinamide was highly effective in reversing the loss of bactericidal activity in both endophytic fungi, as demonstrated by zones of inhibition ranging from 5.03 ± 0.15 – 7.27 ± 0.25 for *Aspergillus* sp. isolate UILRZ5 and from 2.67 ± 0.15 – 7.46 ± 0.50 for *Penicillium citrinum* isolate UILRZ6. In a similar use of epigenetic modifiers by Ameen et al. (2020), the authors noted that endophytic fungi extracts treated with Valproic acid had significantly higher antibacterial activity against *P. aeruginosa* at a concentration of 5 µg/mL. These findings suggest that epigenetic modification is a promising technique for restoring lost bioactivity and can be further explored in future research.

Following successful reactivation, our study examined the impact of combining conventional antibiotics with epigenetically modified endophytic fungi. This approach aimed to evaluate the influence of antibiotics on the antibacterial activity of endophytic fungi. The incorporation of penicillin successfully enhanced the antibacterial activity of both modified fungi. Penicillin may have reactivated silent BGCs in the endophytic fungi, while the fungi's bioactive metabolites may have restored the functionality of penicillin's beta-lactam rings, resulting in the significant activity observed. With penicillin, the *Aspergillus* sp. isolate UILRZ5 exhibited significantly greater activity against the test organisms than the *Penicillium citrinum* isolate UILRZ6. This may be attributed to differences in the synergistic effect between penicillin and the compounds in the endophytic fungal extracts (Al-Fakih and Almaqtri, 2019; Pasrija et al., 2022). To further confirm the effect of the combination therapy, mean MIC trend analysis of the *P. aeruginosa* strains was conducted. The study showed that the combination was more effective at enhancing antibacterial activity than epigenetic modification alone. However, it is important to note that subsequent inferential analyses yielded a different outcome. The ANOVA and Kruskal-Wallis test showed that the MIC values across the extract treatment sets were not statistically significant. Therefore, the evidence presented in this study was insufficient to demonstrate that combining epigenetic modifiers with penicillin increased the antibacterial activity of the endophytic fungi. These results may be due to differences in the test organisms used and variability in the MIC results across the test

Table 16Chemical constituents of nicotinamide-modified *Penicillium citrinum* isolate UILRZ6 extracts.

Peak	Retention time (secs)	Compounds	Area (%)	Formula	Weight (g/mol)
1	6.796	Butane-1,2,3-triol	6.57	C ₄ H ₁₀ O ₃	106
2	6.899	Butane-1,2,3-triol	9.19	C ₄ H ₁₀ O ₃	106
3	7.120	Pantolactone acid	0.67	C ₆ H ₁₀ O ₃	132
4	7.614	Methyl furan-2-carboxylate	2.31	C ₆ H ₆ O ₃	126
5	7.884	3-Nitrobut-3-en-2-ol	0.65	C ₄ H ₇ NO ₃	117
6	8.219	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	12.16	C ₆ H ₈ O ₄	144
7	8.525	1-(furan-2-ylmethyl)pyrrole	1.01	C ₉ H ₉ NO	147
8	8.632	3-hydroxyoctanedioic acid	4.79	C ₈ H ₁₄ O ₅	190
9	8.763	Dianhydromannitol	12.06	C ₆ H ₁₀ O ₄	146
10	8.525	1-Deoxy-D-arabitol	2.87	C ₅ H ₁₂ O ₄	136
11	9.065	Pyridine-2,6-dicarboxylic acid	6.50	C ₇ H ₅ NO ₄	167
12	9.365	2-Oxopentanedioic acid		C ₅ H ₆ O ₅	146
13	9.420	(5-Methylfuran-2-yl)methanol		C ₆ H ₈ O ₂	112
14	9.712	4-Methoxy-4-oxobutanoic acid		C ₅ H ₈ O ₄	132
15	10.613	2-Ethyl-2-methylbutanoic acid		C ₇ H ₁₄ O ₂	130
16	10.931	4-(2-hydroxyethyl)phenol		C ₈ H ₁₀ O ₂	138
17	11.341	4-Ethoxy-4-oxobutanoic acid		C ₆ H ₁₀ O ₄	146
18	11.341	4-Ethylheptan-4-ol		C ₉ H ₂₀ O	144
19	11.751	(2S)-1,1-dimethylpyrrolidin-1-ium-2-carboxylate		C ₇ H ₁₃ NO ₂	143
20	11.341	D-Arabinitol		C ₅ H ₁₂ O ₅	152
21	12.869	Diethyl benzene-1,2-dicarboxylate		C ₁₂ H ₁₄ O ₄	222
22	13.662	3-Hydroxyoctanoic acid		C ₈ H ₁₆ O ₃	160
23	13.853	N-ethyl-N-propylcyclohexanamine		C ₁₁ H ₂₃ N	169
24	14.260	3-Propan-2-yl-2,3,6,7,8,8a-hexahydropyrrolo[1,2- <i>a</i>]pyrazine-1,4-dione		C ₇ H ₁₀ N ₂ O ₂	154
25	14.887	Tetradecanoic acid		C ₁₄ H ₂₈ O ₂	228
26	15.995	2-[3-(2-hydroxyethylamino)-2-methylanilino]ethanol		C ₁₁ H ₁₈ N ₂ O ₂	210
27	16.151	2-[3-(2-hydroxyethylamino)-2-methylanilino]ethanol		C ₁₁ H ₁₈ N ₂ O ₂	210
28	16.537	Methyl hexadecanoate		C ₁₇ H ₃₄ O ₂	270
29	16.613	(E)-Pentadec-2-enoic acid		C ₁₅ H ₂₈ O ₂	240
30	16.665	Diethyl benzene-1,2-dicarboxylate		C ₁₆ H ₂₂ O ₄	278
31	16.796	Hexadecanoic acid		C ₁₆ H ₃₂ O ₂	256
32	17.127	Ethyl nonadecanoate		C ₂₁ H ₄₂ O ₂	326
33	17.521	Methyl (Z)-octadec-9-enoate		C ₁₉ H ₃₆ O ₂	296
34	17.971	Methyl (Z)-octadec-9-enoate		C ₁₉ H ₃₆ O ₂	296
35	18.155	(9Z,12Z)-octadeca-9,12-dienoic acid		C ₁₈ H ₃₂ O ₂	280
36	18.210	Oleic acid		C ₁₈ H ₃₄ O ₂	282
37	18.395	Octadecanoic acid		C ₁₈ H ₃₆ O ₂	284

(continued on next page)

Table 16 (continued)

Peak	Retention time (secs)	Compounds	Area (%)	Formula	Weight (g/mol)
38	18.473	Ethyl Oleate	0.46	C ₂₀ H ₃₈ O ₂	310
39	18.668	Ethyl octadecanoate		C ₂₀ H ₄₀ O ₂	312
40	20.958	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	0.49	C ₂₄ H ₃₈ O ₄	390
41	22.048	2,3-dihydroxypropyl (Z)-octadec-9-enoate	0.90	C ₂₁ H ₄₀ O ₄	356
			0.56		
			100		

groups.

To identify the chemical constituents responsible for the observed antibacterial activity, GC-MS analysis was conducted. The results showed that octadecadienoic acid was detected in the extract of the modified *Penicillium citrinum* isolate UILRZ6. Notably, this compound has demonstrated anti-inflammatory and antiarthritic activity (Kan et al., 2021; Ponnammam and Manjunath, 2012), suggesting that such bioactive compounds contribute to the fungi's antibacterial activity. In contrast, oleic acid, a fatty acid, was able to disrupt bacterial cell walls and inhibit gram-positive bacterial growth (Araki et al., 2017) and was identified in the modified *Aspergillus* sp. isolate UILRZ5 extracts, indicating that its presence may have enhanced antibacterial effects. Furthermore, the detection of several bioactive compounds in epigenetically modified endophytic fungi, compared with unmodified ones, further supports the significance of epigenetic modification as a mechanism for reversing bioactivity. Therefore, this study demonstrates the potential of epigenetic modification as an approach for enhancing the production of secondary metabolites from endophytic fungi. Furthermore, it highlights combinatorial strategies as promising approaches for stimulating the antibacterial potential of these fungi in the search for novel antimicrobial candidates.

5. Conclusion

The endophytic fungi *Aspergillus* sp. isolate UILRZ5 and *Penicillium citrinum* isolate UILRZ6 demonstrate promising antibacterial activity and warrant further research into their bioactivity. Epigenetic modification successfully restored the antibacterial activity of both fungal isolates, and the combination therapy showed synergistic effects with the extracts. GC-MS analyses of both endophytic fungal extracts detected several bioactive compounds, which may have contributed to the renewed bioactivity. Future studies could explore a wider range of microbial strains, epigenetic modifiers, and cultivation conditions to further strengthen evidence for the applications of these approaches. Exploiting epigenetic modification and combination strategies will be invaluable in restoring the bioactivity of endophytic fungi.

CRediT authorship contribution statement

R. F. Zakariyah: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **E. P. Samuel:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **K. A. Ajijolakewu:** Writing – review & editing, Validation, Supervision, Software. **A. S. Ayoola:** Visualization, Resources, Methodology. **R. N. Ahmed:** Visualization, Project administration. **C. O. Olajide:** Project administration, Investigation, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

All datasets generated and analyzed during the study are included within the article. No additional raw data were deposited in external repositories.

References

Abdelgawad, M.A., Hamed, A.A., Nayl, A.A., Badawy, M.S.E.M., Ghoneim, M.M., Sayed, A.M., Hassan, H.M., Gamaleldin, N.M., 2022. The chemical profiling, docking study, and antimicrobial and antibiofilm activities of the endophytic fungi *Aspergillus* sp. AP5. *Molecules* 27, 1704. <https://doi.org/10.3390/molecules27051704>.

Aghamohammad, S., Rohani, M., 2022. Antibiotic resistance and the alternatives to conventional antibiotics: the role of probiotics and microbiota in combating antimicrobial resistance. *Microbiol. Res.* 267, 127275. <https://doi.org/10.1016/j.micres.2022.127275>.

Akram, S., Ahmed, A., He, P., He, P., Liu, Y., Wu, Y., Munir, S., He, Y., 2023. Uniting the role of endophytic fungi against plant pathogens and their interaction. *J. Fungi* 9, 72. <https://doi.org/10.3390/jof9010072>.

Al-Fakih, A.A., Almqatri, W.Q.A., 2019. Overview on antibacterial metabolites from terrestrial *Aspergillus* spp. *Mycology* 10, 191–209. <https://doi.org/10.1080/21501203.2019.1604576>.

Ali, A.H., Reda, D.Y., Ormago, M.D., 2022. Prevalence and antimicrobial susceptibility pattern of urinary tract infection among pregnant women attending Hargeisa Group Hospital, Hargeisa, Somaliland. *Sci. Rep.* 12. <https://doi.org/10.1038/s41598-022-05452-z>.

Ameen, F., Almansob, A., Al Tami, M., Al-Enazi, N., Al-Sabri, A., Orfali, R., 2020. Epigenetic modifiers affect the bioactive compounds secreted by an endophyte of the tropical plant *Piper longum*. *Molecules* 26, 29. <https://doi.org/10.3390/molecules26010029>.

Araki, H., Hajime, M., Tamura, E., Hagihara, H., Takigawa, H., 2017. Human sebaceous cis-6-hexadecenoic acid: possible application of an innate antimicrobial substance to cosmetic products for mucous membrane. *J. Microbiol. Biotechnol.* (<https://www.scrip.org/reference/papers?referenceid=2056994>) (accessed 15 November 2024).

Benite-Ribeiro, S.A., Putt, D.A., Soares-Filho, M.C., Santos, J.M., 2016. The link between hypothalamic epigenetic modifications and long-term feeding control. *Appetite* 107, 445–453. <https://doi.org/10.1016/j.appet.2016.08.111>.

Bertoni, M.D., Levin, L., 1997. Nutritional requirements for growth of an endophyte: *Ceratoprycnidium baccharidicola*. *Rev. Argent. Microbiol.* 29, 131–136. (<https://pubmed.ncbi.nlm.nih.gov/9338922/>).

dos Reis, J.B.A., Lorenzi, A.S., do Vale, H.M.M., 2022. Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. *Arch. Microbiol.* 204, 675. <https://doi.org/10.1007/s00203-022-03283-0>.

Elfadadny, A., Ragab, R.F., AlHarbi, M., F., Badshah, Ibáñez-Arancibia, E., Farag, A., A. Omar, P., Hendawy, Aboubakr, M., Zakai, S.A., Nageeb, W.M., 2024. Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance

- trends, and innovations in breaking therapies. *Front. Microbiol.* 15. <https://doi.org/10.3389/fmicb.2024.1374466>.
- Gundel, P.E., Martínez-Ghersa, M.A., Batista, W.B., Ghersa, C.M., 2010. Dynamics of *Neotyphodium* endophyte infection in ageing seed pools: incidence of differential viability loss of endophyte, infected seed and non-infected seed. *Ann. Appl. Biol.* 156, 199–209. <https://doi.org/10.1111/j.1744-7348.2009.00379.x>.
- Hagag, A.H., Abdelwahab, M.F., Adel, Fouad, M.A., 2022. The endophytic *Aspergillus* strains: A bountiful source of natural products. *J. Appl. Microbiol.* 132, 4150–4169. <https://doi.org/10.1111/jam.15489>.
- Hume, D., Card, S., Rolston, M.P., 2013. Effects of storage conditions on endophyte and seed viability in pasture grasses. 22nd Int. Grassland Congress Adv. seed Sci. Technol. Prod. (https://www.researchgate.net/publication/259368058_Effects_of_storage_conditions_on_endophyte_and_seed_viability_in_pasture_grasses#full-text).
- Jia, M., Chen, L., Xin, H.-L., Zheng, C.-J., Rahman, K., Han, T., Qin, L.-P., 2016. A Friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.00906>.
- Kan, X., Chen, G., Zhou, W., Zeng, X., 2021. Application of protein-polysaccharide Maillard conjugates as emulsifiers: source, preparation and functional properties. *Food Res. Int.* 150, 110740. <https://doi.org/10.1016/j.foodres.2021.110740>.
- Kjærboelling, I., Mortensen, U.H., Vesth, T., Andersen, M.R., 2019. Strategies to establish the link between biosynthetic gene clusters and secondary metabolites. *Fungal Genet. Biol.* 130, 107–121. <https://doi.org/10.1016/j.fgb.2019.06.001>.
- Kulkarni, R.K., Nielsen, B.D., 1986. Nutritional requirements for growth of a fungus endophyte of tall fescue grass. *Mycology* 78, 781. <https://doi.org/10.2307/3807523>.
- Kumari, P., Khan, S., Wani, I.A., Gupta, R., Verma, S., Alam, P., Alaklabi, A., 2022. Unravelling the role of epigenetic modifications in development and reproduction of angiosperms: a critical appraisal. *Front. Genet.* 13. <https://doi.org/10.3389/fgenet.2022.819941>.
- Makhwitine, J.P., Kumalo, H.M., Ndlovu, S.I., Mkhwanazi, N., 2023. Epigenetic induction of secondary metabolites production in endophytic fungi *Penicillium chrysogenum* and GC-MS analysis of crude metabolites with anti-HIV-1 activity. –1404 *Microorganisms* 11, 1404. <https://doi.org/10.3390/microorganisms11061404>.
- Mamangkey, J., Mendes, L.W., Mustopa, A.Z., Hartanto, A., 2024. Endophytic *Aspergillus* and *Penicillium* from medicinal plants: a focus on antimicrobial and multidrug resistant pathogens inhibitory activity. *Biotechnol.* 105, 83–95. <https://doi.org/10.5114/bta.2024.135644>.
- Matúš, P., Littera, P., Farkas, B., Urík, M., 2023. Review on performance of *Aspergillus* and *Penicillium* species in biodegradation of organochlorine and organophosphorus pesticides. *Microorganisms* 11, 1485. <https://doi.org/10.3390/microorganisms11061485>.
- Mdarhri, H.A., Benmessaoud, R., Yacoubi, H., Seffar, L., Assimi, H.G., Hamam, M., Boussettine, R., Filali-Ansari, N., Lahlou, F.A., Diawara, I., Ennaji, M.M., Kettani-Halabi, M., 2022. Alternatives therapeutic approaches to conventional antibiotics: advantages, limitations and potential application in medicine. *Antibiotics* 11, 1826. <https://doi.org/10.3390/antibiotics11121826>.
- Moussa, A.Y., 2024. Endophytes: a uniquely tailored source of potential antibiotic adjuvants. *Arch. Microbiol.* 206. <https://doi.org/10.1007/s00203-024-03891-y>.
- Nazir, A., Puthuveetil, A.R., Hasnain, F., Hamed, K.E., Munawar, N., 2024. Endophytic fungi: nature's solution for antimicrobial resistance and sustainable agriculture. *Front. Microbiol.* 15. <https://doi.org/10.3389/fmicb.2024.1461504>.
- Ngoc, T., Thanh, N.V., 2022. The diversity of endophytic *Aspergillus*. *IntechOpen eBooks*. <https://doi.org/10.5772/intechopen.99717>.
- Omokhua-Uyi, A.G., Gupta, S., Stirk, W.A., van Staden, J., 2025. Antiproliferation and combinatorial antibacterial effects of extracts and endophytes from a South African biotype of *Chromolaena odorata* against β -lactamase producing bacteria. *S. Afr. J. Bot.* 178, 39–49. <https://doi.org/10.1016/j.sajb.2025.01.012>.
- Pasrija, P., Girdhar, M., Kumar, M., Arora, S., Katyal, A., 2022. Endophytes: an unexplored treasure to combat multidrug resistance. *Phytomed.* 2, 100249. <https://doi.org/10.1016/j.phyplu.2022.100249>.
- Ponnammma, S.U., Manjunath, K., 2012. GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. *Int. J. Pharm. Bio Sci.* 3, 570–P576.
- Ramesha, K.P., Chandra Mohana, N., Chandra Nayaka, S., Satish, S., 2021. Epigenetic modifiers revamp secondary metabolite production in endophytic *Nigrospora sphaerica*. *Front. Microbiol.* 12. <https://doi.org/10.3389/fmicb.2021.730355>. (https://www.researchgate.net/publication/286176751_GC-MS_Analysis_of_Phytocomponents_in_The_Methanolic_Extract_of_Justicia_wynaadensis_Nees_T_Anders) (accessed 15 November 2024).
- Rigobelo, E.C., Baron, N.C., 2021. Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology* 13, 1–17. <https://doi.org/10.1080/21501203.2021.1945699>.
- Scorzoni, L., Sangalli-Leite, F., de Lacorte Singulani, J., de Paula e Silva, A.C.A., Costa-Orlandi, C.B., Fusco-Almeida, A.M., Mendes-Giannini, M.J.S., 2016. Searching new antifungals: the use of in vitro and in vivo methods for evaluation of natural compounds. *J. Microbiol. Methods* 123, 68–78. <https://doi.org/10.1016/j.mimet.2016.02.005>.
- Shi, Y.M., Hirschmann, M., Shi, Y.N., Ahmed, S., Abebew, D., Tobias, N.J., Grün, P., Cramés, J.J., Pöschel, L., Kuttelochner, W., Richter, C., Herrmann, J., Müller, R., Thanwisai, A., Pidot, S.J., Stinear, T.P., Groll, M., Kim, Y., Bode, H.B., 2022. Global analysis of biosynthetic gene clusters reveals conserved and unique natural products in entomopathogenic nematode-symbiotic bacteria. *Nat. Chem.* 14, 701–712. <https://doi.org/10.1038/s41557-022-00923-2>.
- Singha, B., Singh, V., Soni, V., 2024. Alternative therapeutics to control antimicrobial resistance: a general perspective. *Front. Drug Discov.* 4. <https://doi.org/10.3389/fddsv.2024.1385460>.
- Toghueo, R.M.K., Boyom, F.F., 2020. Endophytic *Penicillium* species and their agricultural, biotechnological, and pharmaceutical applications. *Biotechnol.* 10, 107. <https://doi.org/10.1007/s13205-020-2081-1>.
- Vázquez-de-Aldana, B.R., Cuesta, M.J., Zabalgoeazcoa, I., 2019. Cultivation and growth dynamics of endophytic fungi in a solid culture medium based on sugar beet pulp. *J. Sci. Food Agric.* 100, 441–446. <https://doi.org/10.1002/jsfa.10030>.
- Weiner-Lastering, L.M., Abner, S., Edwards, J.R., Kallen, A.J., Karlsson, M., Magill, S.S., Pollock, D., See, I., Soe, M.M., Walters, M.S., Dudeck, M.A., 2020. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. *Infect. Control Hosp. Epidemiol.* 41, 1–18. <https://doi.org/10.1017/ice.2019.296>.
- Weng, W., Jiang, S., Sun, C., Pan, X., Xian, L., Lu, X., Zhang, C., 2022. Cytotoxic secondary metabolites isolated from *Penicillium* sp. YT2019-3321, an endophytic fungus derived from *Lonicera japonica*. *Front. Microbiol.* 13. <https://doi.org/10.3389/fmicb.2022.1099592>.
- Werghemmi, W., Abou Fayssal, S., Mazouz, H., Hajjaj, H., Hajji, L., 2022. Olive and green tea leaves extract in *Pleurotus ostreatus* var. florida culture media: effect on mycelial linear growth rate, diameter and growth induction index. *IOP Conf. Ser. Earth Environ. Sci.* 1090, 012020. <https://doi.org/10.1088/1755-1315/1090/1/012020>.
- Wester, A.L., Solberg, S.O., Gopinathan, U., Ahmad, R., Graham, D.W., 2024. Antimicrobial resistance in a one health and one world perspective—Mechanisms and solutions. *Elsevier eBooks*. <https://doi.org/10.1016/b978-0-323-99967-0.00148-4>.
- Zakariyah, R.F., Ahmed, N., Ajijolakewu, K.A., Hamid, A.A., 2025. Enhanced antibacterial potential of fractionated bioactive compounds isolated from endophytic *Nigrospora oryzae* ULRZ1 in *Ocimum gratissimum*. *J. Trop. Biodivers. Biotechnol.* 10, 12019. <https://doi.org/10.22146/jtbb.12019>.
- Zakariyah, R.F., Ajijolakewu, K.A., Ayodele, A.J., Folami-A, B.I., Samuel, E.P., Otuoze, S. O., Abdulrauf, L.B., Ahmed, R.N., 2024. Progress in endophytic fungi secondary metabolites: biosynthetic gene cluster reactivation and advances in metabolomics. *Bull. Natl. Res. Cent.* 48. <https://doi.org/10.1186/s42269-024-01199-x>.